*Proc. Natl. Acad. Sci. USA*
Vol. 81, pp. 2786–2790, May 1984
Cell Biology

# Catabolite repression of chloroplast development in *Euglena*

(two-dimensional/polyacrylamide gels/silver stain/light induced/ethanol)

ANTONIO F. MONROY AND STEVEN D. SCHWARTZBACH*

School of Biological Sciences, University of Nebraska, Lincoln, NE 68588-0118

*Communicated by Myron K. Brakke, January 19, 1984*

ABSTRACT **Two-dimensional gel electrophoresis resolved total cellular protein from *Euglena gracilis* Klebs var. *bacillaris* Cori into 640 polypeptides detectable by silver staining. The addition of 84 mM ethanol to dark-grown resting carbon-starved cells increased the relative amounts of 6 polypeptides and decreased the relative amounts of 3 polypeptides. The addition of 84 mM malate to resting cells increased the relative amounts of 3 of the ethanol-induced polypeptides, suggesting that the induction of these polypeptides represents a generalized response to the provision of a utilizable carbon source, a nutritional shift up, rather than a specific response to ethanol addition. Exposure of dark-grown resting *Euglena* to light increases the relative amounts of 79 polypeptides encoded by the nuclear as well as the chloroplast genome and decreases the relative amounts of 72 polypeptides. Ethanol but not malate specifically inhibited all of the light-dependent changes in polypeptide levels, indicating that chloroplast development in *Euglena* is a catabolite-sensitive process.**

Free-living microorganisms are capable of utilizing a wide variety of organic compounds as the sole source of carbon and energy for growth. The enzymes required to utilize some of these compounds are inducible; their rate of synthesis and steady-state level is significantly increased when the compound they metabolize is present in the environment (1, 2). In eukaryotes, the enzymes comprising some metabolic pathways are localized within subcellular organelles. The induction of microbodies by methanol (3) and ethanol (4), the induction of mitochondria by oxygen (5), and the induction of chloroplasts by light (6) are examples of the induction by a substrate of the enzymes, in this case an entire organelle, required to utilize that substrate. By not forming an organelle under conditions in which it is gratuitous for growth, energy is conserved. In many cases, more than one utilizable carbon source is present in the environment and the presence of one utilizable carbon source represses the synthesis of those enzymes required to utilize an alternative carbon source that is also present. This process, termed catabolite repression (1), establishes a hierarchy of carbon source utilization; a more efficient carbon source will be fully utilized prior to the expenditure of energy that is required to synthesize the enzymes for utilization of the less efficient carbon source. Catabolite repression has been extensively studied in prokaryotes (7) as well as in eukaryotes such as yeast (2, 8), where the synthesis of specific enzymes as well as entire organelles, mitochondria and microbodies, is repressed by glucose, a fermentable carbon source (2).

Just as yeast mitochondria are gratuitous to cells grown on a fermentable carbon source, chloroplasts are gratuitous to cells grown in the dark or in the presence of a utilizable source of organic carbon. Studies with *Chlorella prototheocoides* (9), *Euglena* (10–13), and *Poterochromonas malhamensis* (14) have shown that chloroplast formation is repressed when a utilizable carbon source is present in the medium. In a number of cases, the addition of a utilizable nitrogen source can reverse this repression, leading some authors to suggest that repression is a nonspecific metabolic effect of the carbon/nitrogen ratio in the growth medium rather than a specific metabolic effect of the carbon source (9, 11) and, thus, not an example of catabolite repression of chloroplast biogenesis.

*Euglena* is a facultative phototroph whose heterotrophic growth rate in the dark on a variety of carbon sources such as ethanol or glutamate and malate (6) is comparable with the phototrophic growth rate. Since light, acting through two photoreceptors, induces the formation of the enzymatic machinery required to utilize light and $CO_2$ as the sole source of carbon and energy for growth, a process termed chloroplast development (6), *Euglena* is an ideal organism for studies of the catabolite sensitivity of chloroplast biogenesis. Studies in *Euglena* of light-dependent changes in the specific activity of a number of chloroplast enzymes have shown that ethanol but not malate specifically inhibits chloroplast development in nitrogen-sufficient *Euglena* (12, 13, 15) suggesting that, as found for the development of mitochondria in yeast (2), the development of chloroplasts in *Euglena* is catabolite sensitive. A large number of plant enzymes are, however, activated or inactivated by light (16). Changes in enzyme specific activity could, therefore, be due to ethanol inhibition of enzyme activation rather than catabolite repression of polypeptide synthesis. Furthermore, studies of single enzymes provide no information regarding the extent to which ethanol inhibits light-induced polypeptide accumulation. By using two-dimensional gel electrophoresis, it is possible to overcome these problems associated with studies of changes in the specific activity of individual enzymes. In a single experiment, it is possible to follow changes in the relative amounts of 640 of the most abundant polypeptides of *Euglena* and identify those polypeptides whose relative amount is increased or decreased as a result of light exposure (17). In this paper, we report that ethanol but not malate specifically inhibits all of the light-dependent changes in polypeptide levels; light-induced polypeptide synthesis and degradation, which is controlled by the chloroplast and nonchloroplast photoreceptor, is catabolite sensitive. A brief report of this work has appeared (18).

## MATERIALS AND METHODS

*Euglena gracilis* Klebs var. *bacillaris* Cori maintained in our laboratory in the dark for many years was used. Conditions for cell growth, the preparation of resting cells, light-induced chloroplast development, and the supplementation of resting cells with 84 mM ethanol or 84 mM L-malate have been described (19). Total cellular protein was extracted with phenol and analyzed by two-dimensional gel electrophoresis, and the gels were stained with silver nitrate as described (17). The visualization reaction was stopped 1.5 min after appearance of the first detectable polypeptide by the addition of

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

*To whom reprint requests should be addressed.

0.75 mM NaOH. Sample protein concentrations were determined by the Coomassie blue method (20) using bovine serum albumin as a standard. Only those polypeptides whose relative amounts were altered in at least three out of four independent experiments are reported. The figures represent gels that were run and stained at the same time.

## RESULTS

**Catabolite Induction of Polypeptides.** Two-dimensional gel electrophoresis reproducibly resolves total cellular protein extracted from resting carbon-starved *Euglena* into 640 ± 45 polypeptides that are detectable by silver staining (17). Individual polypeptides are identified by an alphanumeric system (17) consisting of a letter (A–D, A being the most acidic) corresponding to the isoelectric focusing sector of the gel in which the polypeptide is found and a number corresponding to the apparent molecular weight of the polypeptide rounded to the nearest 1000. For proteins of the same molecular weight, a decimal is added with the most acidic protein corresponding to 0.1. The addition of 84 mM ethanol to dark-grown resting cells maintained in the dark reproducibly increased the relative amounts of 6 polypeptides (Fig. 1 *Middle*, diamonds). Three of these polypeptides, A35, B34.1, B34.2, also accumulated when 84 mM malate was added to resting cells (Fig. 1 *Bottom*, diamonds) indicating that, as found for fumarase and succinate dehydrogenase (19), their induction is probably a response of starving cells to a nutritional shift up. Levels of these polypeptides probably decrease during starvation and increase in response to the availability of any utilizable carbon source. The relative amount of 1 of these shift-up proteins, B34.1, decreases when unsupplemented resting cells are exposed to light (17). The remaining 3 ethanol-induced polypeptides, B53, C68, and D87, are induced by only ethanol; their induction represents a specific response to ethanol addition rather than a general response to a nutritional shift up. Polypeptides specifically induced by ethanol are probably enzymes required for ethanol metabolism and many of these polypeptides are localized within glyoxysomes (21, 22). The specific induction of the glyoxysomal marker enzyme, malate synthase, by ethanol is transient (4). Maximal enzyme levels are seen 24 hr after the addition of ethanol to resting cells (4). By 72 hr after ethanol addition, enzyme levels have declined and they are only about 2- to 3-fold higher than in uninduced cells (4). Enzyme levels in the light are also lower than in the dark (4, 23). The small number of polypeptides detected by two-dimensional gel electrophoresis whose levels are reproducibly increased in ethanol-supplemented cells probably reflects the fact that at the time of sampling, 72 hr after ethanol addition, many of the increases in polypeptide levels produced by ethanol, 2- to 3-fold, are at the limits at which a change can be visually detected on the stained gel (17). It is also quite possible that the majority of the glyoxysomal enzymes are not among the 650 most abundant cellular proteins and are therefore not detectable on silver-stained gels.

The addition of 84 mM ethanol to dark-grown resting cells maintained in the dark decreased the relative amounts of three polypeptides; A76, C103.1, and C62.4 (Fig. 1, hexagons). One of these, C103.1, is specifically decreased by ethanol addition; light (17) and malate (Fig. 1) have no effect on the level of this polypeptide. One polypeptide, A76, increases in relative amount on light exposure (17) and ethanol, but not malate, decreased the level of this polypeptide. The other polypeptide whose level decreased after ethanol addition, C62.4 (17), also decreases in unsupplemented cells exposed to light. The addition of malate to resting cells failed to decrease the relative amount of any of the polypeptides resolved.

**Catabolite Repression of Light-Dependent Polypeptide Synthesis.** Light regulates chloroplast development in *Euglena*. Exposure of dark-grown resting *Euglena* to light induces the synthesis of chloroplast-localized enzymes such as NADP-glyceraldehyde-3-phosphate dehydrogenase (12, 24) as well as microbody enzymes such as glycolate dehydrogenase (4, 21). Of the 650 polypeptides resolved by two-dimensional gel electrophoresis, the relative amounts of 79 increase after

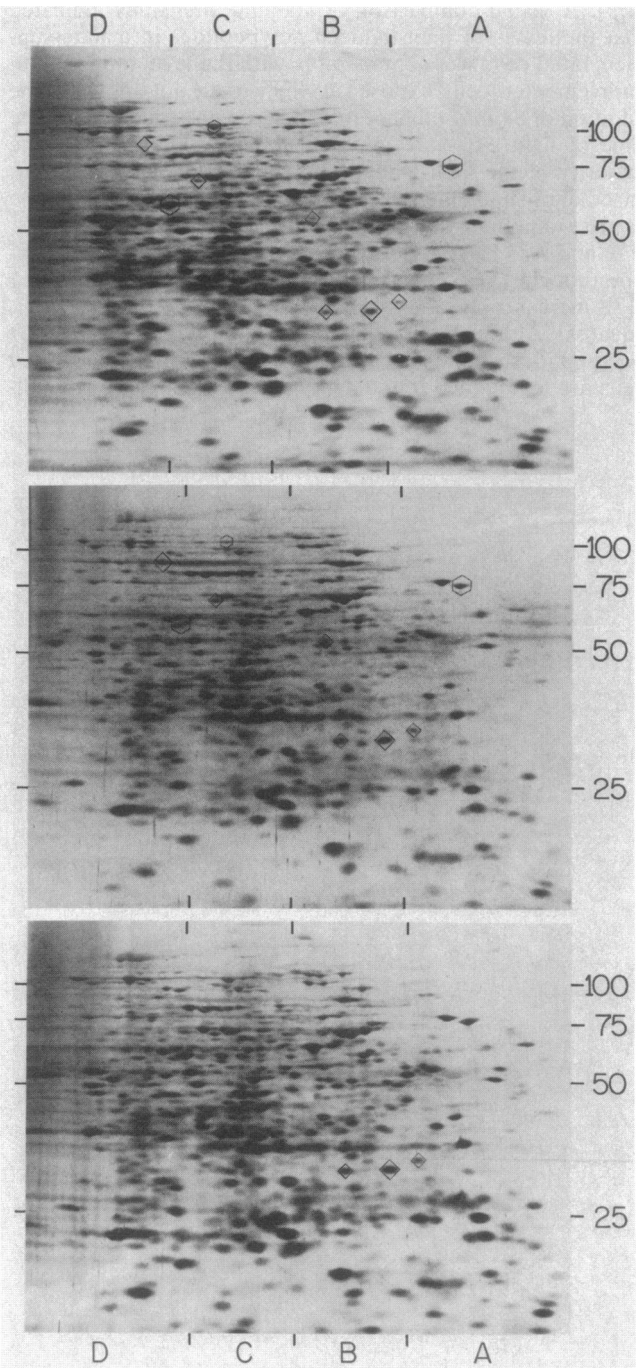


FIG. 1. Ethanol- and malate-dependent changes in the polypeptide composition of *Euglena*. Silver-stained two-dimensional gels of 60 μg of total cellular protein extracted from dark-grown resting *Euglena* maintained in the dark for 72 hr with no additions (*Top*) or in the presence of 84 mM ethanol (*Middle*) or 84 mM malate (*Bottom*) are shown. Polypeptides whose relative amounts decrease or increase on carbon supplementation are enclosed in hexagons or diamonds, respectively. The letters (A–D) indicate the sectors used to identify polypeptides by alphanumeric nomenclature. The numbers on the right indicate approximate molecular weights ($\times 10^{-3}$). Only those polypeptides whose relative amounts increased or decreased in at least three out of four independent experiments are reported.

light exposure (17). Studies with the bleached mutant $W_3$ BUL indicate that at least 12 of those polypeptides are induced by light acting through the blue-absorbing nonchloroplast photoreceptor (17). When 84 mM malate is added to dark-grown resting cells at the time of light exposure, the relative amounts of all 79 light-induced polypeptides increased (Fig. 2 *Top*, squares; compare levels with Fig. 1 *Top*). A visual comparison of staining intensities indicated that the levels of light-induced polypeptides in malate-supplemented cells were comparable with the level found in unsupplemented cells exposed to light (data not shown). The addition of 84 mM ethanol to dark-grown resting cells at the time of light exposure inhibited the photoinduction of the majority of the light-induced polypeptides (Fig. 2 *Bottom*). In ethanol-supplemented cells maintained in the light, the relative amounts of only 2 nuclear-encoded polypeptides, A76 and A23 (17), and the relative amount of one polypeptide thought to be encoded by the chloroplast genome, B51 (17), increased as a result of light exposure (Fig. 2 *Bottom*, squares). Although the level of one of these nuclear-encoded polypeptides, A76, is decreased when ethanol-supplemented cells are maintained in the dark (Fig. 1), its level in ethanol-supplemented cells maintained in the light is higher than its level in dark-grown resting cells prior to ethanol addition. Visual comparison of staining intensities indicates that the level of light-induced polypeptides in ethanol-supplemented cells, however, was always less than the level in unsupplemented cells. The photoinduction of the large subunit of ribulose-1,5-bisphosphate carboxylase/oxygenase (EC 4.1.1.39), polypeptide D54, and two other polypeptides, A17 and D21, thought to be encoded by the chloroplast genome (17), was fully inhibited by ethanol addition, indicating that ethanol inhibits the accumulation of polypeptides encoded by both the nuclear and chloroplast genome. Since malate has no effect on light-induced polypeptide accumulation, repression is a specific effect of ethanol metabolism rather than a general effect produced by the stimulation of cell division and protein synthesis. By analogy to the repression of enzymes of known function, those polypeptides whose induction is fully repressed are probably chloroplast-localized polypeptides such as NADP glyceraldehyde-3-phosphate dehydrogenase (12, 24) and phosphoglycolate phosphatase (25) and microbody-localized polypeptides such as glycolate dehydrogenase (4, 21, 22) that are required for photosynthetic $CO_2$ fixation, while polypeptides whose induction is only partially repressed are probably polypeptides such as chloroplast valyl-tRNA synthetase (13, 15) that are required to maintain the genetic continuity of the chloroplast.

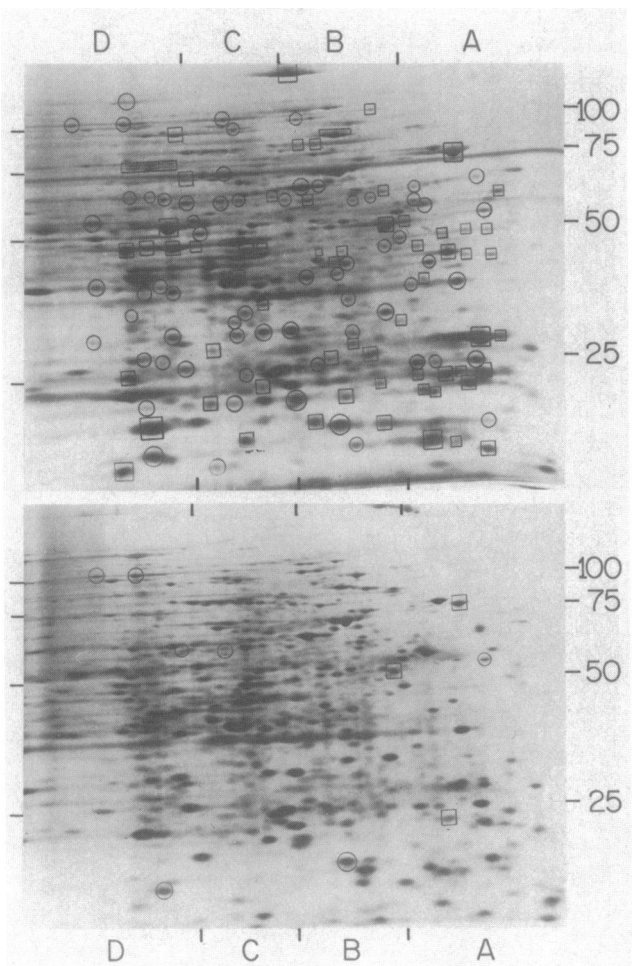


FIG. 2. Catabolite repression of light-dependent changes in the polypeptide composition of *Euglena*. Silver-stained two-dimensional gels of 60 μg of total cellular protein extracted from dark-grown resting *Euglena* exposed to light for 72 hr with addition at the time of light exposure of 84 mM malate (*Top*) or 84 mM ethanol (*Bottom*) are shown. Polypeptides whose relative amounts decrease or increase on light exposure are enclosed in circles or squares, respectively. The letters (A–D) indicate the sectors used to identify polypeptides by alphanumeric nomenclature. The numbers on the right indicate approximate molecular weights ($\times 10^{-3}$). Only those polypeptides whose relative amounts increased or decreased in at least three out of four independent experiments are reported.

A number of polypeptides were induced by the addition of ethanol and/or malate to resting cells maintained in the dark (Fig. 1) and these same polypeptides were induced when the carbon-supplemented cells were exposed to light (Fig. 2). Visual comparison of staining intensities indicated that light exposure had no effect on the levels of ethanol-induced polypeptides. The levels of polypeptides induced by malate addition, however, were lower in cells maintained in the light (compare Figs. 1 and 2).

**Catabolite Repression of Light-Dependent Polypeptide Disappearance.** In resting cells, the amino acids required for chloroplast development are obtained through the degradation of preexisting proteins (12, 24). Thus the specific activities of ALA synthase [succinyl-CoA: glycine *C*-succinyltransferase (decarboxylating), EC 2.3.1.37] (26) and NAD-specific glyceraldehyde-3-phosphate dehydrogenase (EC 1.2.1.12) (24) decrease on exposure of resting cells to light. High levels of these enzymes are required for heterotrophic growth but are gratuitous to phototrophic growth. Of the 640 polypeptides resolved on two-dimensional gels, the relative amounts of 72 polypeptides decrease after 72 hr of exposure to light (17). Studies with the bleached mutant $W_3$ BUL indicate that the disappearance of at least 14 of these polypeptides is controlled by light acting through the blue-absorbing nonchloroplast photoreceptor (17). When 84 mM malate is added to dark-grown resting cells at the time of light exposure, the relative amounts of all 72 light-decreased polypeptides decreased (Fig. 2 *Top*, circles) and the decrease in malate-supplemented cells was comparable with the decrease seen in unsupplemented cells exposed to light (data not shown). The addition of 84 mM ethanol to dark-grown resting cells at the time of light exposure inhibited the light-dependent decrease in the majority of the light-decreased polypeptides. The levels of only 7 polypeptides, A54, B20.2, C62.3, C62.4, D104, D102, and D18, were lower in ethanol-supplemented cells exposed to light than in unsupplemented cells maintained in the dark (Fig. 2 *Bottom*, circles). A visual comparison of staining intensities indicates that the decreases in polypeptide levels that occur in ethanol-supplemented cells exposed to light do not appear to be as great as the decreases seen in unsupplemented cells exposed to light (data not shown). Those enzymes whose light-dependent disappearance is fully inhibited by ethanol are thus probably polypeptides required for heterotrophic growth but gratu-

itous to phototrophic growth. Their degradation provides the amino acids required for the synthesis of chloroplast components (12, 17, 24). Taken together, these results indicate that ethanol at least partially represses all of the light-dependent changes in the actual amounts of *Euglena* polypeptides that are controlled by light acting through a chloroplast and nonchloroplast photoreceptors. The previously reported repression by ethanol of light-dependent changes in the specific activity of a number of chloroplast and nonchloroplast enzymes (10, 12, 13, 25) probably results from a change in the amount of enzyme protein rather than a change in the activity of the enzyme.

## DISCUSSION

Light allows $CO_2$ to be used as a sole source of carbon and energy for growth of *Euglena*. Light induces the enzymes, in this case the entire organelle required for phototrophic growth, a process termed chloroplast development (6). Ethanol can also be used as a sole source of carbon and energy for growth and ethanol induces the formation of glyoxysomes (4, 21), the organelle containing the enzymes required for growth on ethanol (21). Ethanol at least partially inhibits all of the light-dependent changes in polypeptide levels in *Euglena*. Light-dependent increases and decreases in the relative amounts of 151 *Euglena* polypeptides were inhibited when ethanol was added to resting cells at the time of light exposure. This inhibition was not observed when malate was added to resting cells, indicating that the inhibition is a specific effect of ethanol addition rather than a general effect caused by a stimulation of cell division produced by providing a utilizable carbon source to resting (carbon-starved) cells. Glucose also appears to specifically repress the photoinduction of enzymes such as chloroplast valyl-tRNA synthetase (13) whose photoinduction is repressed by ethanol (13, 15). The repression of light-induced enzyme synthesis by glucose and ethanol thus appears to represent the repression by a carbon source of the enzymes, in this case the biogenesis of an entire organelle, required for the utilization of light, an alternative carbon source, a process termed catabolite repression (1). As found for mitochondrial development in yeast (2), catabolite repression of chloroplast biogenesis provides a mechanism to establish a hierarchy of carbon source utilization. In the case of *Euglena*, catabolite repression ensures that energy will be expended to produce the machinery required for the metabolism of ethanol, a carbon source that is not always found in the environment rather than for the metabolism of light and $CO_2$, a carbon source which reappears every 24 hr.

Catabolite repression has been found in both prokaryotes (7) and those free-living eukaryotes that can use a variety of carbon sources for growth (2, 8). In the case of *Escherichia coli*, catabolite repression is mediated by cyclic AMP (7). Transcription of catabolite-sensitive genes is dependent on the binding of a cyclic AMP–cyclic AMP-binding protein complex to the promoter region of catabolite-sensitive genes (7). In the presence of catabolite repressors such as glucose, cyclic AMP levels decrease, the cyclic AMP–cyclic AMP-binding protein complex cannot form and transcription cannot occur due to the absence of this complex (7). In eukaryotes, catabolite repression has been extensively studied in yeast (2, 8). Glucose and other fermentable sugars repress, at the level of gene transcription (2), the synthesis of mitochondrial enzymes, glyoxylate cycle enzymes, and a number of other enzymes that are specifically required for growth on alternative carbon sources (2, 8). In contrast to *E. coli*, in which a single gene controls catabolite repression, multiple genes regulate catabolite repression in yeast (27, 28). Although it has been suggested that catabolite repression in yeast is also mediated by cyclic AMP (2), evidence has accumulated suggesting that cyclic AMP is not involved (8, 29). Thus, in yeast, catabolite repression appears to refer to a number of regulatory systems that regulate the transcription of groups of genes in an unknown manner.

Light regulates the transcription of both the nuclear and chloroplast genomes of *Euglena*. The abundance of some chloroplast genome transcripts is increased while the abundance of other transcripts is decreased as a result of light exposure (30, 31). The complexity of nuclear DNA transcripts is initially increased after light exposure and then declines (32, 33). Transcripts present 24 hr after light exposure are, however, different from those present prior to light exposure (33). The changes in the pattern of gene transcription parallel the changes in the levels of specific polypeptides (17), suggesting that one of the ways in which light regulates the polypeptide composition of *Euglena* is by regulating the pattern of gene transcription.

In *Euglena*, ethanol and acetate induce the synthesis of a number of glyoxysomal enzymes (4, 21–23). The induction of malate synthase by $C_2$ compounds is associated with an increase in translatable mRNA coding for malate synthase (34), indicating that $C_2$ compounds also regulate gene transcription. These same $C_2$ compounds repress chloroplast development (10–13, 15) and our studies with two-dimensional gels indicate that the previously reported repression of the induction, by ethanol, of a large number of enzymes probably results from a decrease in enzyme protein rather than an ethanol-dependent inhibition of enzyme activity. Events controlled by the chloroplast and nonchloroplast photoreceptor are catabolite sensitive. Protochlorophyll(ide) is the chloroplast-localized photoreceptor that regulates gene transcription in *Euglena* (35, 36). Although $C_2$ compounds, ethanol and acetate, repress chloroplast development, they do not inhibit the phototransformation of protochlorophyll to chlorophyll (12, 13) indicating that catabolite repression does not operate at the level of light perception. Since both $C_2$ compounds and light regulate transcription in *Euglena*, it is likely that, as found in yeast and bacteria, catabolite repression results directly from a $C_2$-mediated inhibition of the light-regulated transcription of the nuclear and chloroplast genome. Although cyclic AMP (37) and cyclic AMP-binding proteins (38) have been found in *Euglena*, the level of cyclic AMP has not been shown to be altered by exposing resting cells to light. Since, in *P. malhamensis*, the depletion of glucose from the medium and the initiation of chlorophyll synthesis are associated with changes in the intracellular level of cyclic AMP (14), it will be interesting to determine whether $C_2$ compounds alter cyclic AMP levels in *Euglena*.

This work was supported by National Science Foundation Grant PCM 8202472.

1. Goldberger, R. F., ed. (1979) *Biological Regulation and Development: Gene Expression* (Plenum, New York), Vol. 1, pp. 1–8.
2. Mahler, H. R., Jaynes, P. K., McDonough, J. P. & Hanson, D. K. (1981) *Curr. Top. Cell. Regul.* **18,** 455–474.
3. Fukui, S., Tanaka, A. & Osumi, M. (1975) *Proceedings of the International Symposium on Microbial Growth on $C_1$-Compounds*, Sept. 5, 1974, Tokyo, Japan (The Society of Fermentation Technology, Japan), pp. 139–148.
4. Horrum, M. A. & Schwartzbach, S. D. (1981) *Plant Physiol.* **68,** 430–434.
5. Plattner, H., Salpeter, M. M., Saltzgaber, J. & Schatz, G. (1970) *Proc. Natl. Acad. Sci. USA* **66,** 1252–1259.
6. Schiff, J. A. & Schwartzbach, S. D. (1982) in *The Biology of Euglena*, ed. Buetow, D. E. (Academic, New York), Vol. 3, pp. 313–352.
7. Pastan, I. & Adhya, S. (1976) *Bacteriol. Rev.* **40,** 527–551.
8. Pall, M. L. (1981) *Microbiol. Rev.* **45,** 462–480.
9. Hase, E. (1971) in *Autonomy and Biogenesis of Mitochondria and Chloroplasts*, eds. Boardman, N. K., Linnane, A. W. & Smillie, R. M. (North-Holland, New York), pp. 434–446.

10. App, A. A. & Jagendorf, A. T. (1963) *J. Protozool.* **10,** 340–343.
11. Harris, R. C. & Kirk, J. T. O. (1969) *Biochem. J.* **113,** 195–205.
12. Horrum, M. A. & Schwartzbach, S. D. (1980) *Plant Physiol.* **65,** 383–386.
13. Schimpf, C., Govindarajan, A. G. & Parthier, B. (1982) *Biochem. Physiol. Pflanz.* **177,** 777–788.
14. Handa, A. K., Bressan, R. A., Quader, H. & Filner, P. (1981) *Plant Physiol.* **68,** 460–463.
15. McCarthy, S. A., James, L. & Schwartzbach, S. D. (1982) *Arch. Microbiol.* **133,** 149–154.
16. Buchanan, B. B. (1970) *Annu. Rev. Plant Physiol.* **31,** 341–374.
17. Monroy, A. F. & Schwartzbach, S. D. (1983) *Planta* **158,** 249–258.
18. Monroy, A. F. & Schwartzbach, S. D. (1981) *J. Cell Biol.* **91,** A282 (abstr.).
19. Horrum, M. A. & Schwartzbach, S. D. (1980) *Planta* **149,** 376–383.
20. Spector, T. (1978) *Anal. Biochem.* **86,** 142–146.
21. Graves, L. B., Jr., Trelease, R. M., Grill, A. & Becker, W. M. (1972) *J. Protozool.* **19,** 527–532.
22. Collins, N. & Merrett, M. J. (1975) *Plant Physiol.* **55,** 1018–1022.
23. Cook, J. R. & Carver, M. (1966) *Plant Cell Physiol.* **7,** 377–383.
24. Bovarnick, J. G., Schiff, J. A., Freedman, Z. & Egan, J., Jr. (1974) *J. Gen. Microbiol.* **83,** 63–71.
25. James, L. & Schwartzbach, S. D. (1982) *Plant Sci. Lett.* **27,** 223–232.
26. Beale, S. I., Foley, T. & Dzelzkalns, V. (1981) *Proc. Natl. Acad. Sci. USA* **78,** 1666–1669.
27. Entian, K.-D. (1981) *Mol. Gen. Genet.* **184,** 278–282.
28. Entian, K.-D. & Zimmerman, F. K. (1982) *J. Bacteriol.* **151,** 1123–1128.
29. Thorner, J. (1982) *Cell* **20,** 5–6.
30. Rawson, J. R. Y., Boerma, C. L., Andrews, W. H. & Wilkerson, C. G. (1981) *Biochemistry* **20,** 2639–2644.
31. Chelm, B. K. & Hallick, R. B. (1976) *Biochemistry* **15,** 593–599.
32. Curtis, S. E. & Rawson, J. R. Y. (1979) *Biochemistry* **18,** 5299–5304.
33. Verdier, G. (1979) *Eur. J. Biochem.* **93,** 573–580.
34. Woodcock, E. & Merrett, M. J. (1980) *Arch. Microbiol.* **124,** 33–38.
35. Egan, J. M., Jr., & Schiff, J. A. (1974) *Plant Sci. Lett.* **3,** 101–105.
36. Cohen, D. & Schiff, J. A. (1976) *Arch. Biochem. Biophys.* **177,** 201–206.
37. Keirns, J. J., Carritt, B., Freeman, J., Eisenstadt, M. & Bitensky, M. W. (1973) *Life Sci.* **13,** 287–302.
38. Nicolas, P. & Nigon, V. (1974) *FEBS Lett.* **49,** 254–259.